

Temperature and Relative Humidity Effects on Aerial Exposure Tolerance in the Freshwater Bivalve *Corbicula fluminea*

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Abstract. The exposure tolerance, aerial respiratory behaviors, and the rates of water loss of the Asian freshwater clam, *Corbicula fluminea*, were assessed under three temperature conditions (15°, 25° and 35°C) and five relative humidity (RH) treatments (5%, 33%, 53%, 75% and 95%).

C. fluminea displayed low tolerance to aerial exposure (range of median tolerance times: 23.8–24.9 h at 35°C, 71.4–78.2 h at 25°C, and 248.5–341.6 at 15°C). Relative humidity had no effect on median tolerance time except at 15°C. Body size was reciprocally related to water loss rate at all temperatures, and on longevity at 25° and 35°C. Cumulative rates of water loss at 95% and 75% RH were lower than the other humidities at 15°C, but no differences were found at 25° or 35°C. Mantle edge exposure behavior was inhibited by low humidity and high temperature. Exposing mantle tissues did not increase rate of water loss except at humidities below which the behavior was very rare. The occurrence or extent of the behavior did not affect individual clam longevity. The results suggest that *C. fluminea* can detect rates of desiccation and make behavioral adjustments.

Introduction

The Asian clam, *Corbicula fluminea* (Müller) is a common inhabitant of freshwater habitats in the southern United States (McMahon, 1982, 1983a). Its migration to freshwater is relatively recent and evidence for its estuarine past exists in its higher blood osmolality and differ-

ent ion ratios compared to other freshwater bivalves (Dietz, 1979). The adaptations displayed by *C. fluminea* for survival in freshwater must be recently evolved and may have arisen from estuarine/marine adaptations (Gainey, 1978). Furthermore, the adaptations of corbiculids are derived independently of those of another major freshwater bivalve family, the unionids.

Freshwater bivalve molluscs inhabiting shallow lentic or lotic habitats are subject to periodic emersion as water level drops. Typically, reduced water levels commonly occur during summer months when rainfall is lower and temperatures are higher. Emersion periods are not predictable in their duration or timing. As an adaptation to this stress, some freshwater bivalve species are capable of surviving up to a year out of water (Hiscock, 1953). McMahon (1979) showed that survivability of *C. fluminea* in air is affected by temperature and relative humidity. Associated with emersion are an array of behaviors which include gaping or mantle exposure. This appears to be associated with an aerial respiratory function (McMahon, 1979; McMahon and Williams, 1984). There is evidence that other freshwater bivalves can obtain oxygen directly across the valves (Collins, 1967; Dietz, 1974). Many intertidal marine bivalves maintain aerobic metabolism by continually gaping valves and allowing direct exchange of gasses with the atmosphere (Bayne *et al.*, 1976; Brinkhoff *et al.*, 1983; Shick *et al.*, 1986; for a review see McMahon, 1988). Exposure of large soft tissue surfaces to air should lead to water loss. Under prolonged aerial exposure conditions, *C. fluminea* must balance the advantages of maintaining an aerobic metabolic mode against the requirement to conserve water.

The aims of this study are to investigate the interrelationships of temperature and relative humidity on sur-

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Abbreviations: RH: relative humidity, LSL: log₁₀ shell length, ME: proportion of time spent with mantle exposed.

vivability of *C. fluminea* in air. We examined the effects of body size, and the occurrence and frequency of aerial respiratory behavior on water loss rates under these environmental conditions. We are particularly interested in the interplay between the requirement of preventing dehydration which necessitates valve closure and the metabolic advantages of maintaining contacts with the external environment.

Materials and Methods

Specimens of the Asian clam, *C. fluminea*, for all experiments were collected in June, 1985, from an outflow of Lake Benbrook, Tarrant County, Texas. Animals were maintained in filtered, aerated tapwater at laboratory temperatures (20°–23°C) for at least one week prior to use. A sample of 300 animals was used for the experiments, 20 per relative humidity/temperature combination. Each animal was identified by a lightly etched number on the left valve. The etchings removed the outer periostracal layer to reveal the light colored mineral underneath. It is unlikely that the etchings would have affected gas permeability. Specimens were blotted dry, weighed to the nearest 0.1 mg, and placed onto a desiccator plate in desiccators maintained at the appropriate relative humidity and temperature.

Five relative humidities were chosen, <5%, 33%, 53%, 75%, and >95%. These humidities were maintained in closed desiccators (plate diameter 190 mm) by using silica gel (<5%), supersaturated solutions of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (33%), $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (53%), NaCl (75%), and water (>95%). Salt solutions used to establish the various humidity values are from Wexler and Hasegawa (1954). Relative humidity was measured using a hygrometer (Airguide) and remained $\pm 10\%$ of the starting value during the experiments. Experiments were performed at 15°, 25°, and 35°C ($\pm 0.5^\circ\text{C}$), temperatures chosen to represent a range comparable with those experienced in the environment. Field observations show that individuals of this population when aerially exposed lie just beneath the surface of the sandy substratum, exposing little of the shell. Around 70–80% of the ventral aspect of the valve junction may be exposed. Under these conditions the effects of wind on evaporative loss are reduced and our design, which does not involve air movement, is appropriate.

Animals were reweighed at approximately 3, 6, or 12 hour intervals for 35°, 25°, and 15°C treatments, respectively. Prior to removing animals from the desiccators, the number of individuals exposing the mantle edge, a respiratory behavior, was noted, as were occasions when obvious quantities of fluid had been expelled. At death, the soft tissue was excised, dried to constant weight (>48 h, 90°C) and weighed. The shell was blotted dry and weighed. The experiment continued until all animals were dead.

The total available water [total weight at beginning of the experiment – (wet shell weight + dry tissue weight)], was calculated for each individual. Water loss was expressed as cumulative percent water lost ($100 \times$ weight lost since the start of the experiment/total available water). The points on the curves of the time course of cumulative water loss (Figs 1B, 2B, and 3B) represent values for a standard (grand mean sized) animal of 20.3 mm shell length. Values were derived by performing regression analyses of \log_{10} shell length *versus* cumulative water loss at each sampling interval, and deriving an estimate for a 20.3 mm animal from the regression equation. The number of animals used for the regression analyses (the survivor number) ranged from 20 at the beginning to 3 near the end of the experiments. Of the approximately 150 separate regressions performed, 66% were significant ($P < 0.05$). A significant size relationship to cumulative water loss was more prevalent at 15°C and 25°C (85 and 93% of regressions performed, respectively) than at 35°C (33%). If no significant relationship was indicated ($P > 0.05$) then the mean water loss was utilized.

Hourly rates of water loss were calculated as the percent water lost/sampling interval duration and were categorized as to whether the animal was exposing mantle edge tissues, had closed valves, or had expelled fluid during the sampling interval. Analyses of variance using a repeated measures design on \log_{10} transformed rates were performed, followed by Duncan's multiple range tests to determine differences between temperatures. Analyses of covariance were run with \log_{10} shell length as the covariate and Duncan's multiple range tests were performed to detect relative humidity effects within each temperature. Student's *t*-tests were performed on paired comparisons of water loss rates when valves were closed, when exposing mantle tissues, and when fluid was expelled for each animal at each temperature/relative humidity combination. An index of individual clam behavior is the proportion of time a clam spends with mantle exposed (ME), exclusive of periods during which mantle fluid was expelled. A multiple regression relating clam longevity to relative humidity, \log_{10} shell length, and ME was performed.

Median tolerance times (TL_m or LT₅₀, the elapsed time to 50% mortality) were calculated from the mortality data using the SAS PROBIT procedure (SAS, Cary, NC). Probit analysis transforms the sigmoid time/mortality curve to a linear shape and, by means of linear regression, a mid-point or median value can be derived. Significant differences in median tolerance times were detected by non-overlap of 95% fiducial limits. Other statistical tests also utilized SAS procedures.

Results

There was a significant ($P < 0.05$) temperature effect on median tolerance time (TL_m) (Table 1) resulting in

Table I

Median tolerance times, time elapsed (hours) to 50% mortality as determined by probit analysis, for *Corbicula fluminea* under various conditions of temperature and relative humidity

Temp.	Median tolerance times (hours) relative humidity				
	<5%	33%	53%	75%	>95%
15°C	259.7 A	248.5 A	253.0 A	305.4 B	341.6 C
25°C	72.2 A	71.4 A	73.4 A	78.2 A	75.6 A
35°C	23.8 A	24.0 A	24.0 A	24.4 A	24.9 A

Different letters within a temperature treatment (rows) indicate non-overlap of 95% fiducial limits between those relative humidity treatments.

an approximately threefold increase in TLM with every 10°C decline in temperature. At 15°C, the TLM's at 75% and at 95% RH were significantly different ($P < 0.05$) from one another and both were higher than those at the other relative humidities. No such relationship of relative humidity to TLM was found at either of the other temperature treatments.

During emersion, *C. fluminea* displayed three categories of behavior. The first was simply the closed condition with valves tightly shut, displaying no tissue directly to the environment. The second behavior was that described by McMahon (1979) where the leading edge of the mantle tissue is protruded past slightly gaping valves so that moist tissue is exposed. This mantle edge exposure behavior has been linked to possible aerial oxygen uptake (McMahon and Williams, 1984). Under conditions of lower relative humidity the exposed tissues may appear dry, or a hardened mucus may form between the parted valves. Clams displaying these conditions also were scored as exposing mantle tissues. The third category of behavior was a complete or partial emptying of the mantle cavity water store. This was evidenced at higher humidities by a pool of fluid around the animal. At lower humidities the fluid may have dried, but evidence in the form of dried matter around the animal was an indication that fluid had been expelled.

Mantle edge exposure was influenced both by temperature and relative humidity (Figs 1A, 2A and 3A). High temperature (35°C) inhibited exposure (Fig. 3A), whereas at 25°C and 15°C mantle edge exposure was controlled primarily by relative humidity (Figs 2A and 1A). At 15°C (Fig. 1A) the behavior was observed at every relative humidity treatment. However, the incidence of mantle edge exposure was inhibited at humidities of 33% or lower. At 95% RH, approximately 40% of individuals were exposing tissue at any time. The initial incidence of mantle edge exposure behavior at 75% RH and 15°C was similar to that at 95% RH (Fig. 1A). At 53% RH mantle

edge exposure behavior was reduced and occurred in two bouts, the first during the initial 100 hours of emersion and a second commenced after 200 hours. During these periods few (<20%) individuals were displaying the behavior. The incidence of the behavior was reduced even more at 33% and 5% RH.

At 25°C mantle edge exposure behavior, with one exception, was confined to relative humidities of 53% or greater (Fig. 2A). A distinct correlation existed between the frequency of mantle edge exposure behavior and relative humidity. Fifty to eighty percent of individuals at 95% RH were displaying the behavior between 24 and 54 hours of emersion. During the same period, individuals at 75% RH were exposing mantle 10–40% of the time, while 5–20% of those at 53% RH were exposing tissues.

At 35°C mantle edge exposure behavior was curtailed (Fig. 3A). The occurrences were limited to 53% RH and

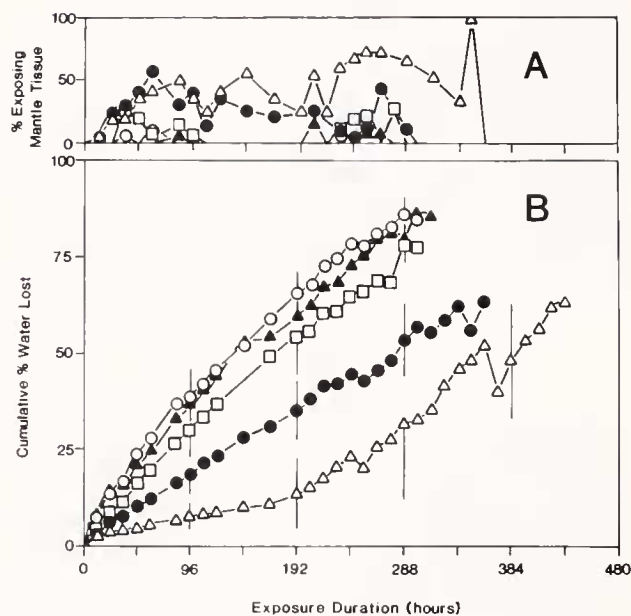


Figure 1. A. Time course of occurrence of mantle edge exposure behavior in *Corbicula fluminea* under five relative humidity treatments at 15°C. Mantle edge exposure behavior is quantified as the percent of individuals observed with mantle protruding at the close of a sampling interval. The abscissal scale is the same as for Figure 1B. Symbols represent \triangle —>95% RH; \bullet —75% RH; \square —53% RH; \circ —33% RH; \blacktriangle —<5% RH.

B. Time course of cumulative percent body water lost for a standard sized (20.3 mm shell length) *Corbicula fluminea* under the same five relative humidity treatments at 15°C as in Figure 1A (symbols the same as Fig. 1A). The points represent estimations of the cumulative water lost for this standard sized animal based on linear regressions of \log_{10} shell length on cumulative percent water lost where these regressions were significant ($P < 0.05$, 85% were significant). Where regressions proved not significant mean cumulative water loss is recorded. The number of animals ranged from 20 at the beginning of the experiment to 3 at the close. The vertical bars are standard errors of the estimate. For clarity, error bars were included at intervals. Declines in cumulative water lost were due to mortality.

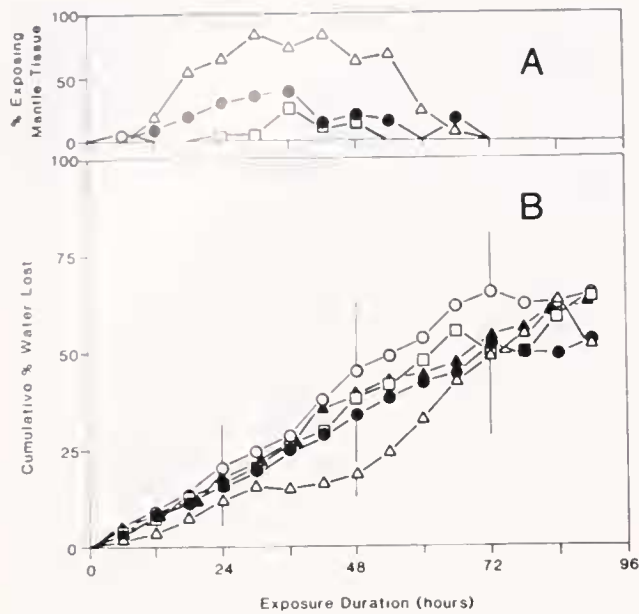


Figure 2. A. Time course of occurrence of mantle edge exposure behavior in *Corbicula fluminea* under five relative humidity treatments at 25°C. Mantle edge exposure behavior is quantified as in Figure 1A. The abscissal scale is the same as for Figure 2B. Symbols represent Δ —>95% RH; \bullet —75% RH; \square —53% RH; \circ —33% RH; \blacktriangle —<5% RH.

B. Time course of cumulative percent body water lost for a standard sized (20.3 mm shell length) *Corbicula fluminea* under the same five relative humidity treatments at 25°C as in Figure 2A (symbols the same as Figure 2A). Methods for calculating curves were the same as in Figure 1B. Approximately 93% of the size-water loss regressions were significant ($P < 0.05$). Declines in cumulative water lost were due to mortality.

above, with only single individuals recorded at 53%. The maximum occurrence was only 30% at 95% RH and less at the lower humidities. The incidences of the behavior were reduced during the period of accelerated water loss (see Fig. 3B), and ceased after 21 hours emersion.

At 15°C the pattern of cumulative water loss showed a distinct relative humidity effect (Fig. 1B). Cumulative water loss values for 5, 33, and 53% RH cluster together and display no differences at any interval. The curves for these treatments are approximately linear throughout the experiment. The curves for 75 and 95% RH are lower than the other relative humidity treatments. The 75% curve was linear whereas the 95% curve showed an initial slow increase followed by an acceleration to a higher loss rate later in the experiment.

At 25°C levels of water loss (Fig. 2B) were 2–6 times those at 15°C. Water loss levels for 5–75% RH were approximately linear and clustered together. At no time during the experiment was any significant difference ($P > 0.05$) found in water loss between the relative humidity treatments.

Cumulative water loss from clams at 35°C (Fig. 3B) was 2–3 times the 25°C values. Water loss was similar for

all relative humidities and no significant differences ($P > 0.05$) were found between treatments. The curves of cumulative water loss were non-linear, showing an increase after 15–18 hours exposure, leveling off at 24 hours as mortality increased.

Overall hourly rates of water loss showed a threefold increase from 15°C to 25°C and a doubling from 25°C to 35°C (Fig. 4). The magnitude of these differences was reduced when rates of water loss during periods of valve closure were considered. When examining rates during both mantle exposure and fluid expulsion, in most cases temperature differences were more pronounced. There were significant differences ($P < 0.05$) between temperature treatments for all relative humidities and behavioral categories except for rates when fluid was expelled between 25° and 35°C at 33% and 53% RH.

No significant differences ($P > 0.05$) in overall rates of water loss existed between 5% and 53% RH treatments at 15°C (Fig. 4). The water loss rates at 75% and 95% RH were significantly less ($P < 0.05$) than the rates at the lower humidities. At 25°C and 35°C no differences in overall mean hourly water loss rates were found between the relative humidity treatments.

When rates of water loss after periods of valve closure

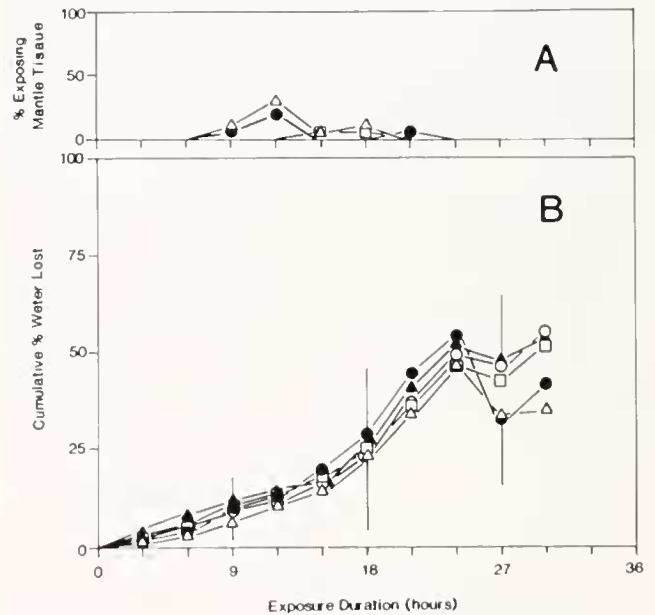


Figure 3. A. Time course of occurrence of mantle edge exposure behavior in *Corbicula fluminea* under five relative humidity treatments at 35°C. Mantle edge exposure behavior is quantified as in Figure 1A. The abscissal scale is the same as for Figure 3B. Symbols represent Δ —>95% RH; \bullet —75% RH; \square —53% RH; \circ —33% RH; \blacktriangle —<5% RH.

B. Time course of cumulative percent body water lost for a standard sized (20.3 mm shell length) *Corbicula fluminea* under the same five relative humidity treatments at 35°C as in Figure 3A (symbols the same as Fig. 3A). Methods for calculating curves were the same as in Figure 1B. Only 33% of size-water loss regressions were significant. Declines in cumulative water lost were due to mortality.

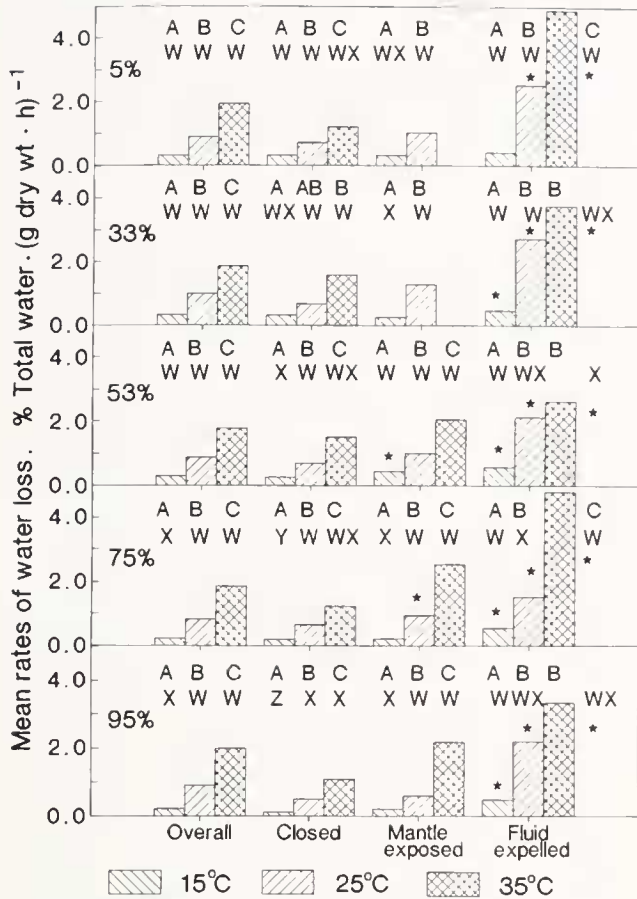


Figure 4. Mean rates of water loss for *Corbicula fluminea* at five relative humidity treatments (5%, 33%, 53%, 75%, and 95%) and three temperatures (15°C, 25°C, and 35°C). Rates of water loss are divided into four behavioral categories (overall average, with closed valves, with mantle exposed, and after fluid expulsion from the mantle cavity; see text). The letters A, B, and C refer to results of Duncan's multiple range tests comparing rates between temperatures, within a relative humidity and behavioral category. Categories marked with dissimilar letters denote significant differences ($P < 0.05$). The letters W, X, Y, and Z refer to results of Duncan's multiple range tests comparing rates between relative humidity treatments, within a temperature and behavioral category. Categories with dissimilar letters denote significant differences ($P < 0.05$). The asterisks denote significant differences ($P < 0.05$; paired 't' test) in mean rates between both the "mantle exposed" and "fluid expelled" behavioral categories and the "closed" category, within each temperature and relative humidity treatment.

(Fig. 4) were examined there were distinct relative humidity effects especially at 15°C. At this temperature the mean rate of water loss at 95% RH was only 40% of the 5% or 33% RH rate. These differences were less apparent at 25°C where the rate at 95% RH was significantly less than the other rates, but represented at most only a 30% reduction. At 35°C there was no consistent pattern of relative humidity effects on mean rates of water loss when valves were closed.

Rates of water loss after periods of mantle edge exposure (Fig. 4) showed no significant differences between

relative humidity treatments at 25°C or 35°C, and no consistent relative humidity effect was found at 15°C. Similarly, rates when fluid was expelled showed no significant ($P > 0.05$) relative humidity effect at any temperature.

No significant differences were found between rates of water loss when valves were closed and rates when mantle edge tissue was exposed at 95% RH at any of the temperatures (Fig. 4). At 15°C when the mantle edge was exposed, the water loss rate was significantly higher than the rate at 53% RH when valves were closed, whereas at 25°C the loss rate when mantle was exposed was significantly higher at 75% RH. Rates of water loss when closed and when exposing mantle tissues were not significantly different at 35°C, at which temperature the behavior of exposing mantle tissues is non-existent at relative humidities below 53%, and is uncommon even at higher humidities. In most cases rates when fluid was expelled were significantly ($P < 0.05$) higher than when closed (Fig. 4). Only the rates at 15°C and 5% RH were not significantly different.

The multiple regression equation relating longevity to relative humidity, \log_{10} shell length, and ME (mantle edge exposure behavior) is shown in Table IIA. Relative humidity had a significant ($P < 0.05$) effect on longevity at 15°C only, as would be expected from the median tolerance time statistics. Animal size had a significant negative effect at both 25°C and 35°C. No significant effect of

Table II

Multiple regression variable estimates relating *Corbicula fluminea* aerial longevity (A) and mean water loss rate while closed (B), to relative humidity (RH), \log_{10} shell length (LSL), and average proportion of time spent with mantle exposed (ME) (longevity only) at 15°, 25°, and 35°C

	Intercept	RH	LSL	ME	R ²
A. Longevity (hours)					
15°C	209.3* (99.3)	0.897* (0.294)	-2.6 (75.6)	21.7 (46.7)	0.22*
25°C	122.1* (26.1)	0.069 (0.060)	-47.1* (20.1)	-2.0 (6.7)	0.08
35°C	41.2* (6.4)	0.006 (0.012)	-17.0* (4.8)	1.5 (3.1)	0.12*
B. Mean water loss rate (valves closed) (% body water · hour ⁻¹)					
15°C	0.959* (0.126)	-0.0021* (0.0003)	-0.462* (0.097)		0.52*
25°C	3.40* (0.39)	-0.0017* (0.0007)	-2.061* (0.304)		0.36*
35°C	4.18* (1.22)	-0.0024 (0.0023)	-2.077* (0.927)		0.06

Values in parentheses are standard errors of estimates. Asterisks indicate estimates significantly different from zero ($P < 0.05$).

mantle edge exposure behavior on clam longevity was noted at any of the temperatures.

Larger individuals lose water at a slower rate than smaller clams as there was a consistently significant ($P < 0.05$) negative effect of body size, as measured by the \log_{10} of shell length, on mean water loss rate when valves were closed at all temperatures (Table IIB). There also was a significant effect of relative humidity at 15°C and 25°C, as would be expected from the results in Figure 4. Relative humidity had a significant effect on water loss after mantle edge exposure at 15°C and 25°C ($P < 0.05$).

Discussion

Temperature is the major factor affecting water loss rate, exposure tolerance, and mantle edge exposure behavior in *C. fluminea* under conditions of prolonged aerial exposure. The effects of relative humidity are less pervasive but significant. Whether water loss is determined as cumulative loss or mean hourly loss rates, relative humidity has little or no effect at 25 or 35°C. At 15°C a distinct mediating effect of higher relative humidities is detected. The major influence of relative humidity is on the incidence of mantle edge exposure behavior. Although temperature is dominant in determining the extent of this response, within a temperature treatment, increases in relative humidity increase the frequency of mantle edge exposure behavior. This indicates that specimens of *C. fluminea* can perceive desiccation and compensate behaviorally. This ability is further evidenced by examining rates of water loss in individuals with closed valves and after bouts of mantle edge exposure. Under conditions of high relative humidity there is no difference in rates of water loss within a temperature group. Significant increases in water loss rate occur only at relative humidities where mantle edge exposure behavior is transitional between being commonplace and being rare. Thus, under conditions where there are no adverse consequences of exposing tissues with regard to losing water, then the behavior is common. When water loss rates exceed rates when the animal has closed valves, then the behavior is inhibited.

The occurrence of a second period of mantle edge exposure behavior at the lower humidities suggests additional factors become important. When clams had lost 60–70% of body water, which corresponds to the total mantle cavity water store plus some hemolymph water (McMahon, 1979) a second bout of mantle edge exposure behavior is observed. In all cases this behavior is displayed in individuals close to death and might represent a loss of muscle tone associated with a weakened condition. Another possibility is that it represents a final gas exchange event prior to complete shutdown to conserve water.

Clams that expose tissues extensively do not survive

longer than those that remain closed for extended periods. Furthermore, there is no relative humidity effect on median tolerance time at 25°C even though mantle edge exposure is significantly enhanced by higher relative humidity. Thus the adaptive significance to mantle edge exposure is obscure. McMahon and Williams (1984) showed a direct relationship between duration of mantle edge exposure behavior and aerial oxygen uptake. Other experiments (Byrne, unpub.) show that clams prevented from gaping their valves in air have a lower median tolerance time than those permitted to expose tissues (at 20°C and 95% RH the TLM is 154 hours when allowed to open valves, and is only 96 hours with valves prevented from opening, $P < 0.05$).

There are several adaptive explanations of mantle edge exposure behavior. First, it is possible that gas exchange necessary for the maintenance of aerobic metabolism may be accomplished with little exposure of tissues. The measurement of mantle edge exposure utilized here is essentially a "snapshot" of behavior. There is evidence (Byrne, unpub.) that clams, in addition to the more constant mantle edge exposure behavior also perform valve movements which ventilate the mantle cavity. Such behaviors are relatively short in duration and could be missed. Second, being out of water, there would be a buildup of metabolic waste products including dissolved CO₂ and concomitant acid-base problems as a result of reduction in gas exchange capability. Intermittent mantle edge exposure may aid gas exchange but may not significantly alter hemolymph solute concentrations, which might be the cause of the reduced survival time. Under aerial exposure conditions, blood osmolality increases threefold and calcium levels rise fourfold indicating mobilization of calcium carbonate from the shell (Byrne, unpub.). Third, the behavior may conserve energy stores. Even the most energy "efficient" anaerobic pathways are far less efficient than aerobic metabolism (Hochachka and Somero, 1984). Although the extent of the mantle edge exposure did not increase the survival time in air, the animal might have been conserving limited energy stores by maintaining aerobic metabolism. Whereas aerial survival would not be influenced by mantle edge exposure, subsequent abilities to resume aquatic existence would be enhanced in individuals that conserved substrates (McMahon, 1988).

The effect of body size on the ability to survive aerial exposure is two sided. There is a distinct reciprocal relationship between size and water loss rate. However, at 25 and 35°C there also is a negative relationship between individual clam longevity and size. The temperature effect is most severe at 35°C whereas size-dependent effects on water loss rates are significant at all temperatures. Thus, under short term conditions of aerial exposure, larger clams would have an adaptive advantage over smaller clams because of the larger clam's slower

rate of water loss. The effect of body size is not significant after periods of mantle edge exposure suggesting that evaporative water loss under these conditions is a simple function of the linear size of the exposed mantle, an almost two-dimensional surface, rather than being related to a surface area of the whole clam while valves are closed.

When compared to other freshwater bivalves, *C. fluminea* displays a low tolerance of aerial exposure. McMahon (1979) showed a somewhat longer aerial exposure tolerance for *C. fluminea* than reported here, but also found that relative humidity had an effect on median tolerance time at the lower temperature (20°C). Mantle edge exposure behavior was inhibited completely at the lower humidity (approximately 5% RH), but was common at the higher humidity (ca. 95% RH). The unionid *Ligumia subrostrata* could survive up to 40 days exposure under conditions of high relative humidity at temperatures of 22°–25°C (Dietz, 1974). An Australian unionid, *Hyridella australis*, apparently is capable of withstanding at least 60 days of exposure at room temperatures (Hiscock, 1953). *Sphaerium occidentale*, the fingernail clam, can survive aerial exposure for up to a month; the survival time is related to relative humidity (Collins, 1967). This species undergoes a period of aestivation during which the tolerance to aerial exposure is increased (McKee and Mackie, 1980). Non-aestivating *S. occidentale* has a median tolerance time at 20°C of 1–3 days, and aestivating clams 8–34 days, the range controlled by relative humidity. The record for exposure endurance is held by the African unionid, *Aspatharia petersi*, which has been reported to survive over a year out of water (Dance, 1958).

The lower tolerance of *C. fluminea* to exposure is similar to that of more marine/estuarine species. Median tolerance times for *Cerastoderma edule* at high relative humidity range from 129 h at 15°C to 9.5 h at 35°C (Boyden, 1972). These values are lower than those of *C. fluminea*, but represent an extended tolerance when compared to less-often exposed marine bivalves. *Cerastoderma glaucum*, found subtidally, has TLM's of 86.7, 42, and 9.7 h at 15, 25, and 35°C, respectively (Boyden, 1972). *Cerastoderma edule* gapes almost continuously when emersed and can survive 42.9% of tissue water loss, whereas *C. glaucum* remains closed on exposure and can withstand only 33% tissue water loss (Boyden, 1972). However, the high intertidal bivalve *Modiolus modiolus* air gapes while emersed and has a TLM of 9 days in air at 24.5°C (Lent, 1968). Survival time is associated with the volume of oxygen present in the atmosphere. Desiccation is an unavoidable side effect of an aerial respiratory adaptation (Lent, 1968).

Pulmonate snails represent a group of molluscs that have adapted to a range of habitats from completely terrestrial to obligate aquatic (McMahon, 1983b). Length

of survival in air depends on the rate of water loss from the snail (Machin, 1975). Tolerance of a high loss of body water results in increased tolerance times. *Biomphalaria glabrata* can withstand 70% reduction in total body water and has a TLM of 64 days at 27°C at 96% RH (von Brand *et al.*, 1957). There are sharp distinctions of TLM between relative humidity treatments ranging down to 2.8 days at 15% RH (von Brand *et al.*, 1957). *Melampus bidentatus* can lose up to 78.5% of its body water (Price, 1980) and there is a major effect of body size in that larger snails can withstand in excess of 14 days exposure at 20°C and 97% RH whereas juvenile snails cannot survive 12 hours emersion without free water.

C. fluminea when aerially exposed under conditions of low humidity may form a hardened mucus between slightly parted valves. This is superficially similar to the epiphragm sometimes produced by estivating stylomorphoran snails which is thought to function in retarding evaporative water loss by reducing convective movement of air over moist tissues (Machin, 1975). The hardened mucus may perform a similar function in *C. fluminea* while also allowing a diffusive contact with the environment for gas exchange purposes.

It has been noted previously (McMahon, 1979, 1983a; McMahon and Williams, 1984) that *C. fluminea*, because of its relatively recent history in freshwater, displays physiological and behavioral adaptations that seem to be intermediate between those of more ancient freshwater forms such as the unionids and sphaeriids, and its estuarine and marine relatives. Its blood osmolality is higher, and the major hemolymph ions differ from those of unionids (Dietz, 1979). McMahon and Williams (1984) suggested that the respiratory adaptation to aerial exposure is intermediate between the continual gapping of some intertidal bivalves (*e.g.*, *Cerastoderma edule*) and the closed valve response of unionids, or the transvalvular gas exchange capabilities of some sphaeriids. This intermediate nature also is evidenced in this study by the lower exposure tolerances found for this species when compared to most other freshwater bivalves, and the higher tolerance than that reported for most of estuarine/intertidal lamellibranchs.

There are two major problems facing a clam that has been exposed in air. First, there is the problem of maintaining metabolism and homeostasis without the normal modes of waste excretion, gas exchange, and ion regulation. Second, there is the loss of fluid either by passive surface evaporation or by active expulsion. Loss of nutritional opportunities frequently is regarded as not very significant owing to the overriding importance and immediacy of the other factors. However, in *C. fluminea*, carbohydrate stores are limited (Williams and McMahon, 1985) and prolonged emersion might cause severe carbohydrate depletion. Preserving water stores necessitates maintaining closed valves and preventing contact

with the external environment. Ameliorating internal conditions in the face of declining oxygen tensions, increasing carbon dioxide tensions, and increasing concentrations of metabolites requires some gas exchange with the environment and the consequent exposure of living tissues to a more or less arid atmosphere.

Corbicula fluminea possesses adaptations which enable it to survive periods of emersion longer than its estuarine relatives. Apparently *C. fluminea* can detect the loss of water and modify its gas exchange behavior accordingly. It can withstand a loss of up to 60% of its available water; however, it does not display the capabilities of some unionid species to withstand very long periods of aerial emersion. Unlike the marine and estuarine environments, freshwater animals may experience periods of emersion that are unpredictable either in timing or duration. Some unionid clams, with their long history in freshwater, appear to have evolved adaptations enabling them to withstand protracted exposure. *C. fluminea* in its own adaptations shows interesting modes of prolonging existence out of water. These adaptations, including the modification of the "gaping" behavior in response to temperature and relative humidity may be regarded as intermediate in nature between those of more ancient freshwater and estuarine forms, but might also be looked upon as novel adaptations to a new environment by a relatively recent invader.

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